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Introduction

In many areas of Louisiana, wetlands have subsided to open water habitat characterized by depths greater than 0.5 m. Due to waterlogging stress, most of these areas no longer support emergent vegetation (Penfound and Hathaway 1938, DeLaune et al. 1978, Gornitz et al. 1981, Buamann and Day 1984, Mitsch and Gosselink 1993). Furthermore, these open-water areas continue to increase in coastal Louisiana at a rate of three acres each hour (or roughly 35 square miles per year, Boesch et al. 1994). In attempt to redress this loss, several sediment diversion and hydrologic restoration projects have been implemented or are planned under the Coastal Wetlands Planning, Protection, and Restoration Act (CWPPRA 1993). Nevertheless, many areas of Louisiana are either isolated from potential sediment sources or are not suited for such projects because of the need for maintaining navigable waterways. These areas will continue to degrade unless a mechanism for site-specific sediment addition is devised.

One such method involves using restored drill cuttings, a by-product of the oil and gas industry, to build elevations suitable for colonization and establishment of wetland vegetation. Through physical isolation of metals and organics in a silica matrix and removal of toxic constituents with stabilizing agents, the cuttings are restored to acceptable levels (Swaco Geolograph, New Orleans). Although cuttings from a single drilling project would yield perhaps less than one-acre of emergent wetland, cumulatively such projects could add significantly to current restoration activities. Certainly, if restored sediments can be shown to cause no environmental hazards, support wetland vegetation, and have a restoration cost comparable to current disposal costs, then this method warrants serious consideration.

During the last year, with support from the Department of Energy and Southeastern Louisiana University's College of Arts and Sciences, a state-of-the-art mesocosm facility was constructed and a program was implemented to determine the efficacy of creating wetlands with restored drill cuttings under three hydrologic regimes. Two drill cuttings processing methods (Cameron and Swaco) were assessed along with a dredge spoil substrate (which capped the Cameron substrate) and a topsoil (control). The initial results are very interesting and are presented herein.

Materials and Methods

Experimental Approach

Design. One hundred forty four 200-liter vessels, fully networked to four 3000-liter supply vessels, were subjected to a 3 x 4 x 6 factorial treatment arrangement with two true replicates per treatment combination (detailed in Appendix A). Specifically, three hydrologic regimes, four substrates, and six vegetative conditions were applied in a factorial arrangement as described below.

Hydrologic Regimes. Three hydrologic regimes were established. The three hydrologic regimes consisted of moist-but-not flooded, permanently flooded, and daily tidal cycle conditions. The moist-but-not flooded treatment was maintained by leaving the low tide drain in the open position and trickling supplemental moisture into the vessel during the high tide cycle. Permanent flooded conditions were maintained at a depth of 20 cm above the sediment surface by setting the low tide drain in the closed position and the high tide drain in the open position. The daily tidal cycle regime was achieved by on-off switches controlled by timers and resulted in a flood-tide depth of 20 cm above the sediment surface and an ebb-tide depth of -10 cm below the sediment surface. Tides were based on a lunar day of 24.8 hours, thereby resulting in a tidal lag of approximately 0.8 hours later on each subsequent day, as occurs in nature.

Substrate Types. The drill cuttings used in this project were generated in Grand Bay, Louisiana. The raw cuttings were then treated via two processes. First, they were treated with the Cameron process designed to separate and recycle drilling muds (lubricants) from drill cuttings. This process decreases the weight of material to be transported to a hazardous waste facility, thereby decreasing transportation and waste costs. A second process, termed the Swaco process, further remediates the drill cuttings via physical isolation of metals and organics in a silica matrix. Any remaining toxic agents are then diluted with stabilizing agents. Treatment of the cuttings occurs until constituents reach stabilized, acceptable (Louisiana Department of Natural Resources (LaDNR) No. 29-B) levels. Six cubic yards each of cuttings that had undergone treatment with the Cameron and Swaco processes, plus six cubic yards of dredge spoil, were transported by dump truck to the mesocosm facility at Southeastern Louisiana University, courtesy of Greenhill Petroleum Corporation. In a completely cross-classified manner, one hundred and forty four 200-liter vessels were filled by hand with the following substrates: (1)

topsoil, (2) Cameron, (3) Swaco, and (4) Cameron capped with 40 cm of dredge spoil. The dredge spoil cap treatment was included in case the vegetation failed to establish directly on the cuttings material, and to enable direct comparison of the restored cuttings material with the substrate currently being used in wetlands creation projects in coastal Louisiana (i.e., dedicated dredging projects, CWPPRA 1993). Rarely would the roots of herbaceous wetland vegetation penetrate below a 30 cm depth (Mitsch and Gosselink 1993).

Vegetation. Six vegetation conditions were established across the hydrologic regime and substrate type combinations. Individual plants of each species were collected in the field and rinsed of all marsh soil, and planted during June, 1996. One of the species, arrowhead (*Sagittaria latifolia*), had zero survival and was subsequently replaced with elephantsear (*Colocasia esculenta*). The other five vegetative conditions were bulltongue (*Sagittaria lancifolia*), maidencane (*Panicum hemitomon*), two treatments of wiregrass (*Spartina patens*, six genotypes isolated by Dr. Hester which have been shown to demonstrate low, intermediate, and high stress tolerance to elevated salinity levels, planted as separate treatments with three of each tolerance type per treatment), and the unvegetated control. *Sagittaria* species tend to be fresh marsh pioneer species (Godfrey and Wooten 1979). Chabreck (1972) reported that *Panicum hemitomon* and *Sagittaria lancifolia* are the dominant fresh marsh emergent macrophytes in Louisiana. *Spartina patens* is a wide-spread brackish marsh dominant and is the most frequently encountered coastal grass species in Louisiana (Chabreck 1972; Godfrey and Wooten 1979). *Colocasia esculenta* is a wide-spread fresh to intermediate marsh species (Godfrey and Wooten 1979).

Data Analysis. The data were analyzed using SYSTAT 6.0 statistical software (SYSTAT Inc. 1996). Photosynthetic response was originally analyzed as a split-plot design, with peak growing season and end of growing season placed in the subplot, along with all interaction pertaining to time. The time effect was highly significant ($F = 104.7$, $P < 0.0001$) indicating that photosynthetic response behaved differently during the two periods. Therefore, each time period was analyzed separately as a completely randomized design with a 3 x 4 x 6 factorial treatment arrangement.

Variables Measured

Plant photosynthetic response, biomass partitioning, and elemental analysis of plant tissue, sediment and sediment interstitial water were determined as described below.

Plant Photosynthetic Response. Instantaneous measurements of plant photosynthetic response (net CO_2 assimilation and stomatal conductance) were conducted during August and again October, 1996 using a LICOR 6400 portable photosystem. Two measurements were obtained from each of three individual stems per mesocosm vessel under uniform, light saturated conditions and net CO_2 assimilation expressed as μmol of CO_2 fixed per m^2 per second.

Biomass Partitioning. In early November aboveground biomass was harvested at the sediment surface and partitioned into live and dead components. Harvested tissue was then oven-dried until constant weight was achieved and weighed. Belowground biomass will remain intact for further analysis of vegetative regrowth during year two.

Elemental Analysis. For all treatment combinations, sediment, sediment interstitial water, and water from the 3000-liter reservoirs were collected for elemental analysis and nutrient analysis of NO_3/NO_2 and NH_4 nitrogen on two sampling dates. Plant tissue was collected at harvest for elemental analysis.

Acid digestion of each substrate were conducted on dried samples that were homogenized with a mortar and pestle and then digested in concentrated HNO_3 at 130° C and subjected to the spectrophotometer for elemental analysis. Water extraction was conducted by placing 10 g of dried substrate samples in centrifuge tubes and extracting with 30 ml of distilled water while shaking for one hour. The extract was then filtered and subjected to elemental analysis. Plant tissue elemental analysis was performed on dried (live) tissue similarly digested in concentrated nitric acid at 130° C. The plant tissue analysis is currently underway and will be presented in the year two final report.

Elemental analysis was conducted on acid preserved (with HNO_3) samples analyzed with a Jarrel-Ash inductively coupled argon plasma - optical emission (ICP-OES Atom Comp Series 800, later referred to as ICP) spectrophotometer for concentrations of Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, and Zn. For the nutrient analysis, samples were immediately frozen and are currently undergoing analysis with a Technicon Auto Analyzer.

Toxicity Trials. Drill cuttings treated by the two restoration methods were used in 96-hour Static Definitive Toxicity trials (U.S. EPA, Federal register, Vol. 58, No. 41, 1993 (40 CFR Part 435/12507)) by Environmental Enterprises, Slidell, LA. Testing was conducted using mysid shrimp (*Mysidopsis bahia*) and the suspended particulate phase (SPP) of the drill cuttings. The mysid shrimp were 3 to 6 days old, cultured and maintained in 23 ppt ($\pm$ 2) and 23° C ($\pm$ 2) and fed a daily ration of fairy shrimp (*Artemia* sp.) nauplii. The shrimp were acclimatized to a salinity of 20 ppt ($\pm$ 1) and a temperature of 20° C ($\pm$ 2) prior to initiation of the toxicity trials. Standard reference toxicant (95 % pure sodium dodecyl sulfate, Sigma Chemical) tests yielded a 96-hour LC_{50} of 7.5 ppm, with a 95 % confidence interval of $\pm$ 0.60. A drill cuttings-seawater slurry, mixed at a 1:9 ratio, was shaken for two hours and allowed to settle (EPA 1993). After one hour, the suspended particulate phase (SPP) was decanted and monitored for pH, temperature, dissolved O_2, and salinity. When necessary, pH and dissolved O_2 were adjusted (EPA 1993). Sixty mysids (three replicates of twenty) were exposed to five SPP concentrations and controls. Surviving mysids were counted and recorded at 24-hour intervals, and measurements made of temperature, dissolved O_2, pH, and salinity.

Results

Elemental Analysis

Results of acid digestion elemental analysis conducted on drill cuttings from Swaco and Cameron processing, dredge spoil, sediment from the proposed wetland creation site, and a composite sample of unprocessed raw cuttings (i.e., a homogenized sample of the raw cuttings taken at various depths from the well) prior to the initiation of the experiment are presented in Tables 1 and 2. It is important to note that acid digestion is a measure of the total potentially extractable elements, not what would be extracted under normal environmental conditions. The last column (Table 2) displays the 29-B standards of LaDNR; none of the substrates exceeded these 29-B limits. However, the Swaco-processed substrates are quite alkaline (pH = 10, Table 2). A subsequent water extraction elemental analysis of the Swaco and Cameron substrates (Table 2) indicates that elements that are high in the cutting material tend to be tightly bound to the substrate as corresponding concentrations in the water extract are generally several orders of magnitude lower than their acid digestion extract counterpart. The cuttings, regardless of whether they were processed with the Swaco or Cameron treatments, tended to be high in iron, calcium, and magnesium, none of which cause damage to wetlands (in fact, iron augmentation may be beneficial). The two processes differ in that the Swaco restoration treatment contains high levels of aluminum. The corresponding high pH is almost certainly associated with an abundance of aluminum hydroxides (Mengel and Kirby 1987).

Two months following initiation of the study, the interstitial water of dredge spoil was more similar to the drill cuttings material than to topsoil, particularly with regard to cations (Table 3). For example, potassium in topsoil was 14.6 ppm compared to 34.4, 41.9, and 48.1 ppm in Cameron, Swaco, and dredge spoil, respectively. Furthermore, topsoil sodium concentrations were 94.5 ppm compared to 912.9 (Cameron), 702.6 (Swaco), and 1276.1 ppm (dredge spoil). Calcium concentrations were similar between dredge spoil and topsoil (31 to 33 ppm), whereas calcium concentration was slightly higher in Cameron (50.8 ppm) and lowest in Swaco (2.6 ppm). Aluminum concentrations were low to non-detectable in Cameron, dredge spoil, and topsoil, but remained elevated in Swaco (Table 3). These patterns in elemental differences between substrates in interstitial water two months into the study are similar to the initial analyses (Table 1, 2). As in the initial analysis, none of the analyzed elements exceeded the limits set by LaDNR 29-B standards. In summary, the elemental makeup of Cameron drill cuttings is more similar to dredge spoil than Swaco, and is generally as conducive as dredge spoil in supporting healthy wetland plant growth (see below). One hundred and forty eight (144 vessels plus the four reservoirs) end of the growing season samples are currently undergoing analysis and will be included in the year two final report, as will nutrient analyses.

<u>Toxicity Trials</u>

Baseline toxicity limits are established at 30,000 ppm (EPA 1993), indicating that SPP concentrations causing toxicity below 30,000 ppm are deemed toxic, whereas those above that threshold are considered safe. The 96-hour exposure to 6 %, 13 %, 25 %, and 100 % suspended particulate phase concentrations resulted in an LC_{50} of 639,700 ($\pm$ 71,000, 95 % confidence limit) for Swaco and greater than 1,000,000 (the upper limit of detection) for the Cameron treated cuttings. Survival in the 100 % SPP was 90 % in both substrates, compared to 100 % survival in the Control. These results are extremely promising with regard to the low toxicity of restored drill cuttings.

Photosynthetic Response - Peak Growing Season

Hydrologic Regime. The main effect of variable hydrologic regime on photosynthetic rate was not statistically significant, but is shown (Figure 1) because the trend depicted agrees with wetland ecosystem theory (Mitsch and Gosselink 1993). Stagnant conditions, as would occur during the first year of the field study, initially yielded the lowest rates of photosynthesis, while the tidal throughput treatment was characterized by the highest levels of photosynthesis, across all plant species. This may be important if the levees of the pending wetland creation site are eventually breached in the future.

With respect to photosynthetic rate (net CO_2 assimilation) during peak growing season, the interaction between hydrologic regime and vegetative species was significant (Figure 2, $F = 2.36$, $P = 0.015$) indicating that the species responses were not consistent across hydrologic regimes. For example, maidencane (Figure 2) demonstrated a dramatic increase in net CO_2 assimilation not seen in the other species in the tidal hydrologic regime.

Plant Species. The clones of wiregrass (*Spartina patens*), a plant that utilizes the C_4 photosynthetic pathway, acheived the highest rates of photosynthesis (linear contrast of wiregrass vs. other plant species (i.e., mudflat omitted) produced $F = 179.74$, $P <0.0001$). Interestingly, all of the species included in the project, across substrates, fixed CO_2 at moderate to high rates (Figure 3, $F = 29.79$, $P < 0.0001$).

The interaction between plant species and substrate type (Figure 4, $F = 3.60$, $P < 0.0001$) is primarily attributable to the mudflat response of zero photosynthesis. This needs to be interpreted with caution, because a dense mat of benthic algae was visible on several mudflat mesocosm vessels (especially those processed by Swaco), but the photosystem is not designed to measure microalgal productivity. Fortunately, the principal investigator has designed a modification to the photosystem that will enable measurement of algal productivity during year two of the project.

With respect to stomatal conductance (a measure of water lost from a plant) during the peak growing season, the interaction between species and substrate was significant (Figure 5, F =

2.56, P = 0.003). Along with the species by substrate interaction for photosynthetic response (Figure 4), this indicates that all genotypes of wiregrass (*Spartina patens*) maximized the overall photosynthetic response in the Cameron substrate. In this substrate, wiregrass net CO_2 assimilation was high and the stomatal conductance was low, a combination that is physiologically ideal for the overall photosynthetic response, because it results in high water-use efficiencies (high carbon assimilation per amount of water lost).

Substrate Effect. During the peak growing season, perhaps the most striking effect was the main effect of substrate, both for net CO_2 assimilation (Figure 6) and stomatal conductance (Figure 7). Net CO_2 assimilation of the Cameron and topsoil treatments did not differ statistically ($F = 0.05$, $P = 0.828$), and together the two substrates produced significantly higher photosynthetic rates (linear contrast produced $F = 47.19$, $P < 0.0001$) than the other substrates. It is noteworthy that stomatal conductance (a measure of water loss) was significantly lower in the Cameron substrate when compared with the topsoil control ($F = 48.89$, $F < 0.0001$), indicating that the Cameron-restored drill cuttings result in high plant water-use efficiencies and therefore appear well suited for wetlands creation projects (as long as some other factor does not limit plant growth).

Photosynthetic Response - End of Growing Season

Hydrologic Regime. In contrast to peak growing season, plant photosynthetic response showed no trend with respect to hydrologic regime ($F = 0.228$, $P = 0.80$). However, photosynthetic response displayed a significant interaction between hydrologic regime and vegetative condition ($F = 2.11$, $P = 0.03$, Figure 8), indicating that species differences existed with respect to sensitivity to hydrologic regime.

Plant Species. The main effect of vegetative condition was highly significant ($F = 84.99$, $P < 0.0001$, Figure 9). A prior contrasts indicated that wiregrass had greater photosynthetic rates than all other plant species ($F = 248.7$, $P < 0.0001$). Similarly, within the grass species, wiregrass produced greater photosynthetic rates than maidencane ($F = 185.2$, $P < 0.0001$). Together the grass species (maidencane and wiregrass) had greater photosynthetic rates than the forbs (bulltongue and elephantsear). Nonetheless, the forbs had greater photosynthetic rates than maidencane by itself. A significant interaction between vegetative condition and substrate type was also detected for photosynthetic response ($F = 4.46$, $P < 0.0001$, Figure 10). As expected, for many of the species the topsoil treatment produced the highest photosynthetic rates. However, for wiregrass topsoil performed similarly to dredge spoil and the Cameron drill cuttings. In fact one group of wiregrass genotypes performed equally well across all substrates. In general, all other species (and the other group of wiregrass genotypes) had the lowest photosynthetic rates in the Swaco drill cuttings. Nevertheless, all species tested had positive net CO_2 assimilation rates in the Swaco cuttings.

Substrate Effect. The main effect of substrate type was highly significant ($F = 17.8$, $P <0.0001$, Figure 11). As expected, the topsoil substrate had significantly higher photosynthetic rates than all other substrates ($F = 37.8$, $P < 0.0001$). Importantly, the Cameron drill cuttings supported higher rates of photosynthesis than the Swaco cuttings ($F = 4.3$, $P = 0.040$). Although the photosynthetic rates of plants grown on Cameron appeared similar to those grown on dredge spoil, a contrast revealed that dredge spoil slightly outperformed Cameron ($F = 4.3$, 0.042). An interaction between substrate type and hydrologic regime was significant ($F = 2.54$, $P = 0.025$, Figure 12). Under moist or flooded conditions, topsoil had the greatest photosynthetic followed by a consistent decrease from dredge spoil to the Swaco substrate. However, under tidal conditions, topsoil, dredge spoil and Cameron substrates all resulted in similar high rates of photosynthesis that were greater than those of the Swaco substrate.

Aboveground Biomass

Hydrologic Regime. Plant aboveground biomass displayed no significant main effects or interactions with regard to hydrologic regime.

Plant Species. The species effect was significant ($F = 152.4$, $P < 0.0001$), as was the interaction of species with substrate type ($F = 15.4$, $P < 0.0001$, Figure 13). In all species, the Swaco drill cuttings yielded the poorest biomass production. The forbs (bulltongue and elephantsear) yielded the greatest biomass when grown on the topsoil substrate. Interestingly, wiregrass had the greatest biomass when grown on either Cameron or dredge spoil and actually displayed a slight decrease when grown on topsoil (Figure 13). However, maidencane had depressed biomass production on all substrates except topsoil.

Substrate Type. The main effect of substrate on total aboveground biomass was highly significant ($F = 127.6$, $P < 0.0001$, Figure 14). When averaged across species, topsoil produced the greatest aboveground biomass ($F = 205.2$, $P < 0.0001$). Dredge spoil and Cameron, which did not differ from each other ($F = 0.05$, $P = 0.82$), yielded the second greatest aboveground biomass across species. Furthermore, Swaco drill cuttings resulted in significantly less aboveground biomass than Cameron ($F = 127.0$, $P < 0.0001$, Figure 14).

Discussion

The results obtained thus far are promising with regard to the low toxicity of restored drill cuttings (particularly the Cameron substrate) and their ability to support healthy wetlands vegetation. Water extraction, acid digestion, and interstitial water samples from the restored drill cuttings all yielded elemental analyses that fell within the LaDNR 29-B guidelines. This is particularly encouraging since acid digestion represents the worst case scenario of total potentially extractable elements upon complete digestion of the substrate, a situation highly not representative of nature.

The Cameron drill cuttings are remarkably similar to dredge spoil which is currently being used as a wetlands creation substrate (i.e., dedicated dredging, CWPPRA 1993). The few elements that were extracted into the interstitial water were primarily cations (Ca, K, Mg) and were not elevated to a level that would pose a threat to wetlands productivity. Swaco drill cuttings remained high in aluminum with concomitant high pH, which likely resulted in limited plant productivity through hindered nutrient uptake (Larcher 1995). One potential negative aspect of the Cameron drill cuttings is elevated electrical conductivity (Table 2). However, other than the response of maidencane (restricted to fresh marsh), the other plant species tested did not appear to be severely affected. In particular, wiregrass (widespread coastal marsh grass) performed nearly as well on Cameron substrate as dredge spoil.

The toxicity trial results based on mysid shrimp showed that both Swaco and Cameron had acceptable toxicity levels (LC_{50}) at all percentages of suspended particulate phase (6% to 100%). Survival in the 100% suspended particulate phase was 90% in both substrates, further demonstrating the low toxicity of these restored drill cuttings.

Plant photosynthetic responses showed interesting trends due to hydrology during the peak growing season, with the tidal regime tending toward the highest rates. However, photosynthetic responses measured at the end of the growing season no longer displayed a trend in hydrologic regime. This may be due to temperature buffering in the permanently flooded treatments, thereby resulting in a delayed onset of dormancy. Results obtained from year two of the study will yield important information concerning the mitigating effect of season on plant responses to hydrologic regime. Nonetheless, it is important that the permanently flooded hydrologic regime supported healthy growth in most of the species tested because the pending field demonstration project will be impounded to restrict hydrologic exchange with the surrounding wetlands for at least one year.

Cameron drill cuttings supported higher rates of photosynthesis, across species, than Swaco drill cuttings. Biomass measured at the end of year one showed that average biomass production on the Cameron substrate was essentially identical to that on the dredge spoil (Figure 14). This finding is important in documenting the suitability of Cameron drill cuttings for wetland restoration because plant growth response is an integrated indicator of a species cumulative stress response (Osmond et al. 1987, Ewing et al. 1995, Larcher 1995, Bazzaz 1996). Figure 15 further documents the similarity between Cameron and dredge spoil substrates by showing the non-significant differences between aboveground biomass least-squared means. Although Figure 15 also documents the reduced plant production on the Swaco substrate, it is important to note that all substrates tested resulted in positive net CO_2 assimilation rates and hence biomass accumulation.

Wiregrass appears extremely promising for stabilizing wetlands restored with drill cuttings. Wiregrass had the highest overall photosynthetic rates across substrates and exceeded other species on both drill cuttings substrates. During year two genotype differences in performance will be elucidated. Bulltongue and elephantsear also appear suitable as species that can be established on restored drill cuttings with resultant high biomass production. All three of these species (wiregrass, bulltongue, and elephantsear) produced similar amounts of biomass on Cameron-restored drill cuttings as on dredge spoil (Figure 13). Although none of the species tested performed well on the Swaco-restored cuttings, bulltongue and elephantsear outperformed wiregrass on this substrate in terms of biomass production. Maidencane only performed well on the topsoil treatment and appears unsuitable for use in wetlands restoration projects that utilize drill cuttings or dredge spoil. This plant is restricted to fresh marshes and prefers organic soils

(Chabreck 1972). Arrowhead, a species that did not survive the initial planting, also appears unsuitable for this type of project, at least during summer transplantings since it is very sensitive to handling during the warmer months.

Results from this first year have clearly demonstrated highly variable species responses to establishment and performance on restored drill cuttings, as well as dredge spoil and topsoil. Species differences will be further assessed during year two, in which we will also conduct soil seed bank studies, transplant another species in place of maidencane, and transplant baldcypress seedlings into the bare mudflat vessels.

In summary, results from year one of this project have yielded several interesting findings. Most importantly, the Cameron-restored drill cuttings have a low toxicity and are capable of supporting several wetland plant species at levels of biomass production directly comparable to dredge spoil. It is important to note that the mesocosm facility has enabled emulation of the worst-case scenario (that is the scenario most likely to transfer elements from the substrate to the water column), namely a closed tidal system with subsurface extraction of recycled interstitial water. Even under these extreme conditions the restored drill cuttings appeared to be non-toxic and supported vigorous vegetative biomass production. In short, results from this mesocosm project indicate that a field demonstration project utilizing restored drill cuttings is safe and will likely result in the creation of healthy and stable wetlands.

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Table 1. Elemental analysis results (µg/g in solution) from substrate and interstitial water taken from the two substrate types (SWACO and Cameron drill cuttings at time zero.

	Swaco Substrate	Swaco Water	Cameron Substrate	Cameron Water
[Cu]	58.074	0.336	24.897	0.553
[Zn]	61.679	0.068	52.057	0.032
[Cd]	2.487	0.000	1.349	0.000
[Pb]	0.000	0.000	0.000	0.000
[Cr]	32.688	0.846	24.492	0.000
[Ni]	26.056	0.000	16.932	0.060
[As]	32.181	0.324	33.423	0.000
[Fe]	244418.391	9.316	17651.393	2.329
[Mn]	177.655	0.030	262.189	0.000
[Ca]	78576.975	1099.704	8457.614	136.397
[Mg]	17507.533	3.612	5674.877	30.827
[P]	692.442	0.396	318.008	2.338
[Al]	38546.589	145.037	2129.811	1.800
[K]	1462.362	71.036	3734.085	76.493
[Ba]	1310.470	0.708	226.476	0.199
[Ag]	124.535	1.120	41.546	0.668

Table 2. Elemental analysis results (μg/g of substrate) for the SWACO and Cameron drill cuttings, dredge spoil, the field site, and a composite of raw cores ranging from a depth of 1000 feet to 9000 feet below the surface of the earth, at time zero.

	SWACO	Cameron	Dredgespoil	Field Site	Composite	Standards Not to Exceed
[Zn]	63.800	60.600	76.300	64.300	56.900	500 ppm
[Cd]	2.000	0.700	1.300	1.100	1.100	10 ppm
[Pb]	25.500	14.700	17.200	21.400	13.900	50 ppm
[Cr]	41.100	17.500	18.500	15.800	18.500	500 ppm
[As]	2.100	ND	ND	0.900	3.000	10 ppm
[Ba]	14500.000	12500.000	ND	ND	4500.000	20000 ppm
[Ag]	ND	ND	ND	ND	ND	200 ppm
[Hg]	ND	ND	ND	ND	ND	10 ppm
[Se]	ND	ND	ND	ND	ND	10 ppm

ND -- Non Detectable Level   All Above Measurements in μg/g   ppm = parts per million

						Units
Electrical Conductivity	6.94	26.10	7.31	Not Available	1.44	mmhos/cm
Exchangeable Sodium	43.0	115.90	52.30	Not Available	10.80	%
Cation Exchange Capacity	16.0	22.00	23.00	Not Available	17.00	meq/100g
Soil pH	10.11	8.83	7.94	Not Available	8.20	St. units

Table 3. Elemental analysis results (ppm in solution) from the interstitial water analysis of composite samples taken from the four substrate types (SWACO and Cameron drill cuttings, dredge spoil, and topsoil) two months after the study was initiated

Elements	SWACO	Cameron	Dredge Spoil	Topsoil
[Cu]	0.029	0.017	0.000	0.011
[Zn]	0.000	0.000	0.000	0.000
[Cd]	0.000	0.000	0.001	0.000
[Pb]	0.000	0.005	0.000	0.000
[Cr]	0.059	0.000	0.000	0.000
[Ni]	0.000	0.017	0.008	0.001
[As]	0.002	0.000	0.000	0.000
[Fe]	0.000	0.023	0.000	0.000
[Mn]	0.000	0.000	0.000	0.000
[Ca]	2.555	50.842	32.737	30.992
[Mg]	0.000	23.150	97.398	5.800
[P]	0.162	2.576	0.000	0.020
[Al]	16.494	0.106	0.000	0.000
[K]	41.941	34.116	48.133	14.643
[Mo]	0.326	0.053	0.011	0.000
[Na]	702.646	912.906	1276.136	94.503
[S]	203.328	291.971	309.516	25.053
[Si]	0.000	13.299	0.000	0.548
[Co]	0.000	0.001	0.001	0.001

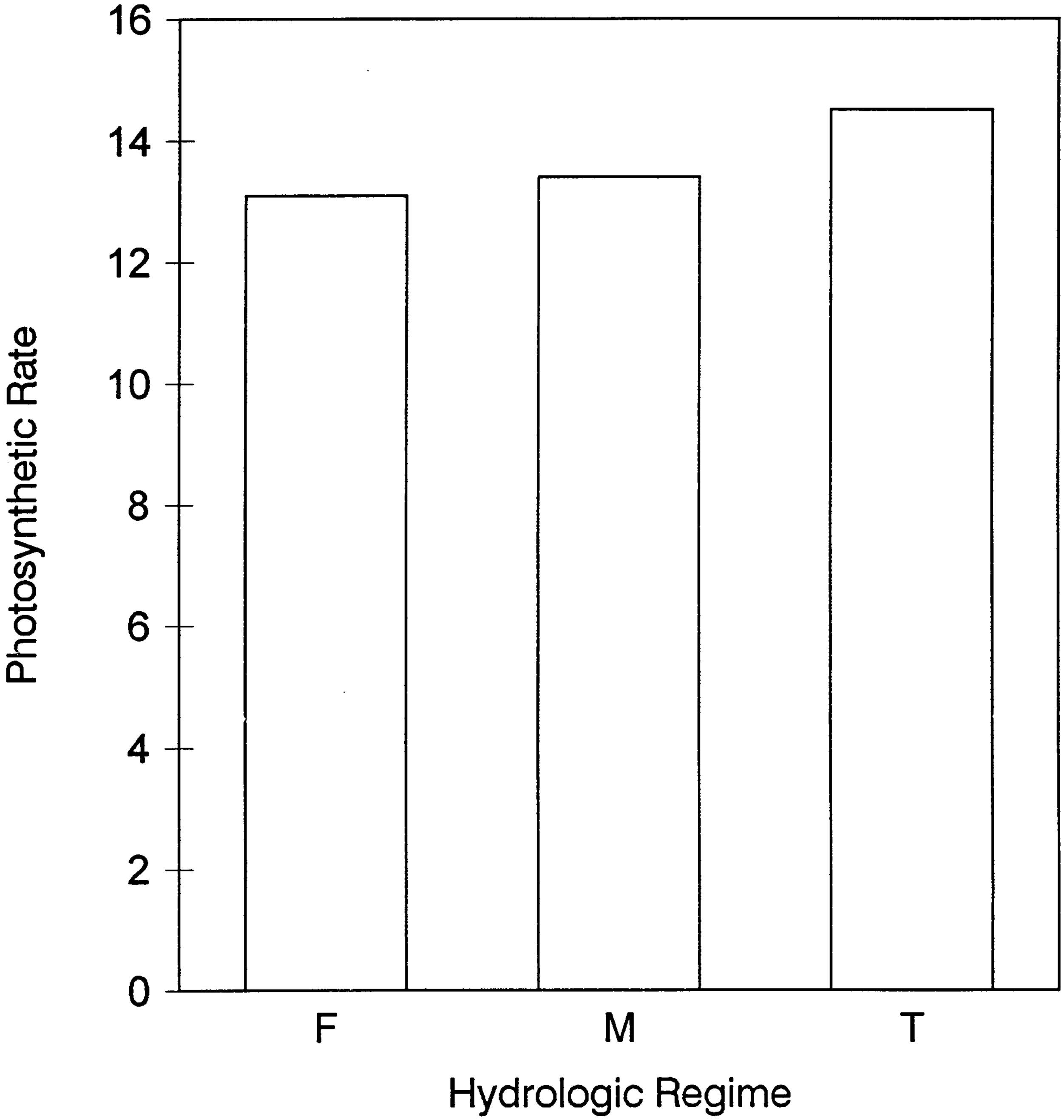
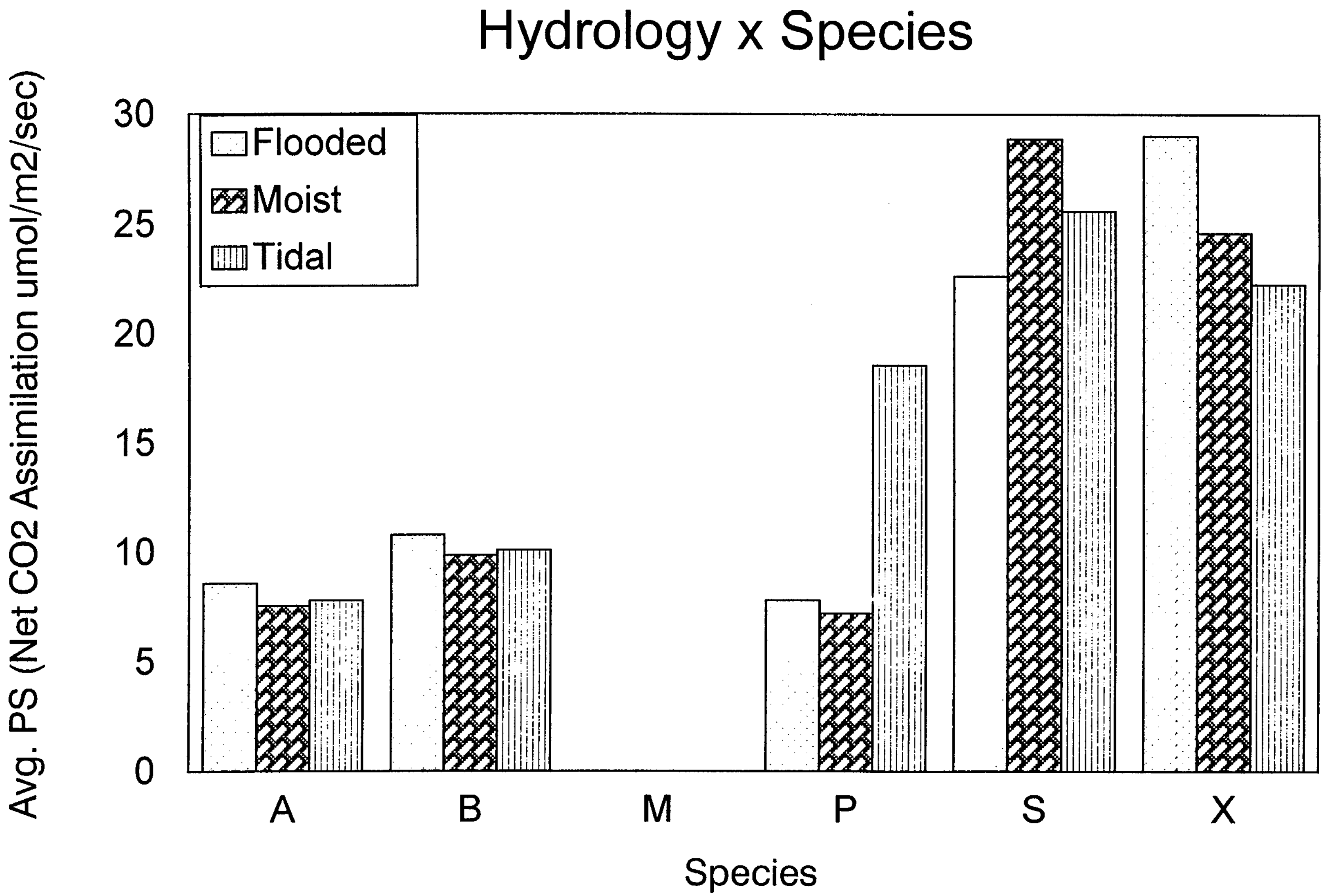


Figure 1. Photosynthetic rate for various hydrologic regimes (F = flooded permanently, M= moist-but-not flooded, T = tides daily).

Figure 2. Interacting effects of hydrologic regime and species (A = elephantsear, B = bulltongue, M = mudflat, P = maidencane, S = average of three genotypes of wiregrass, X = average of the other three genotypes of wiregrass) on photosynthetic rate.



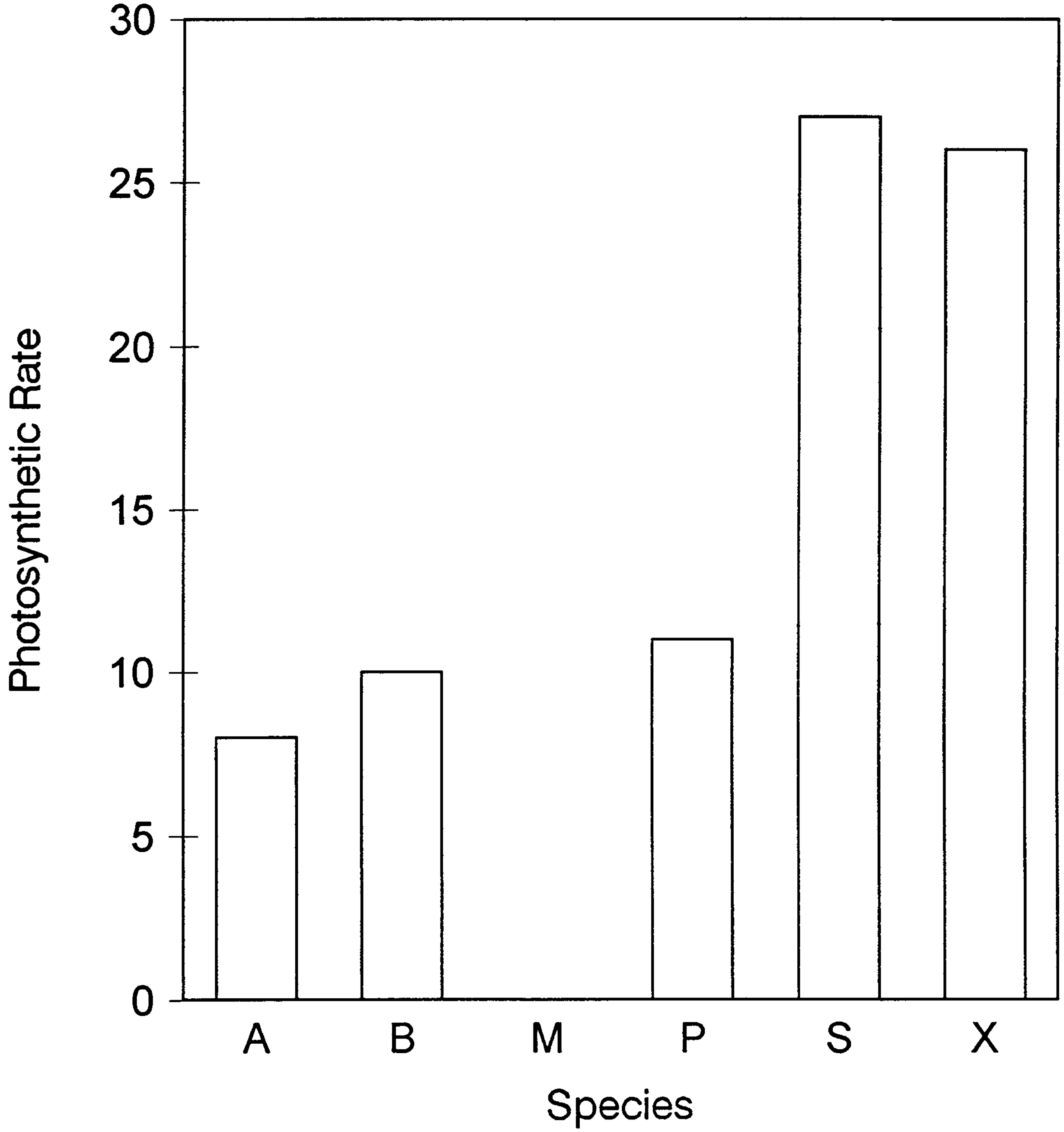


Figure 3. Average photosynthetic response for the six vegetative types (A = elephantsear, B = bulltongue, M = mudflat, P = maidencane, S = average of three genotypes of wiregrass, X = average of other three genotypes of wiregrass).

Figure 4. Interaction of plant species (A = elephantsear, B = bulltongue, M = mudflat, P = maidencane, S = average of three genotypes of wiregrass, X = average of the other three genotypes of wiregrass) and substrate type on photosynthetic response.

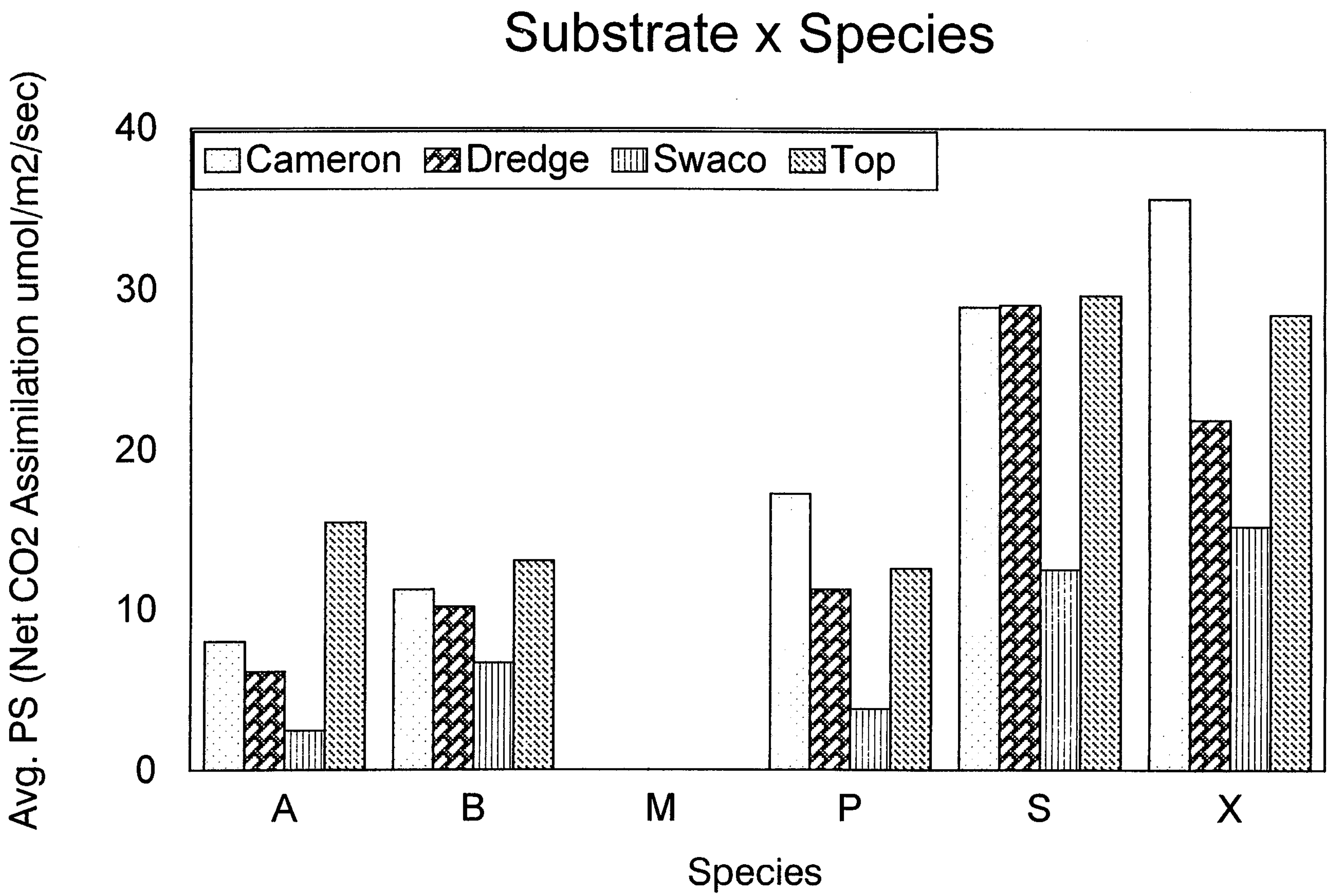
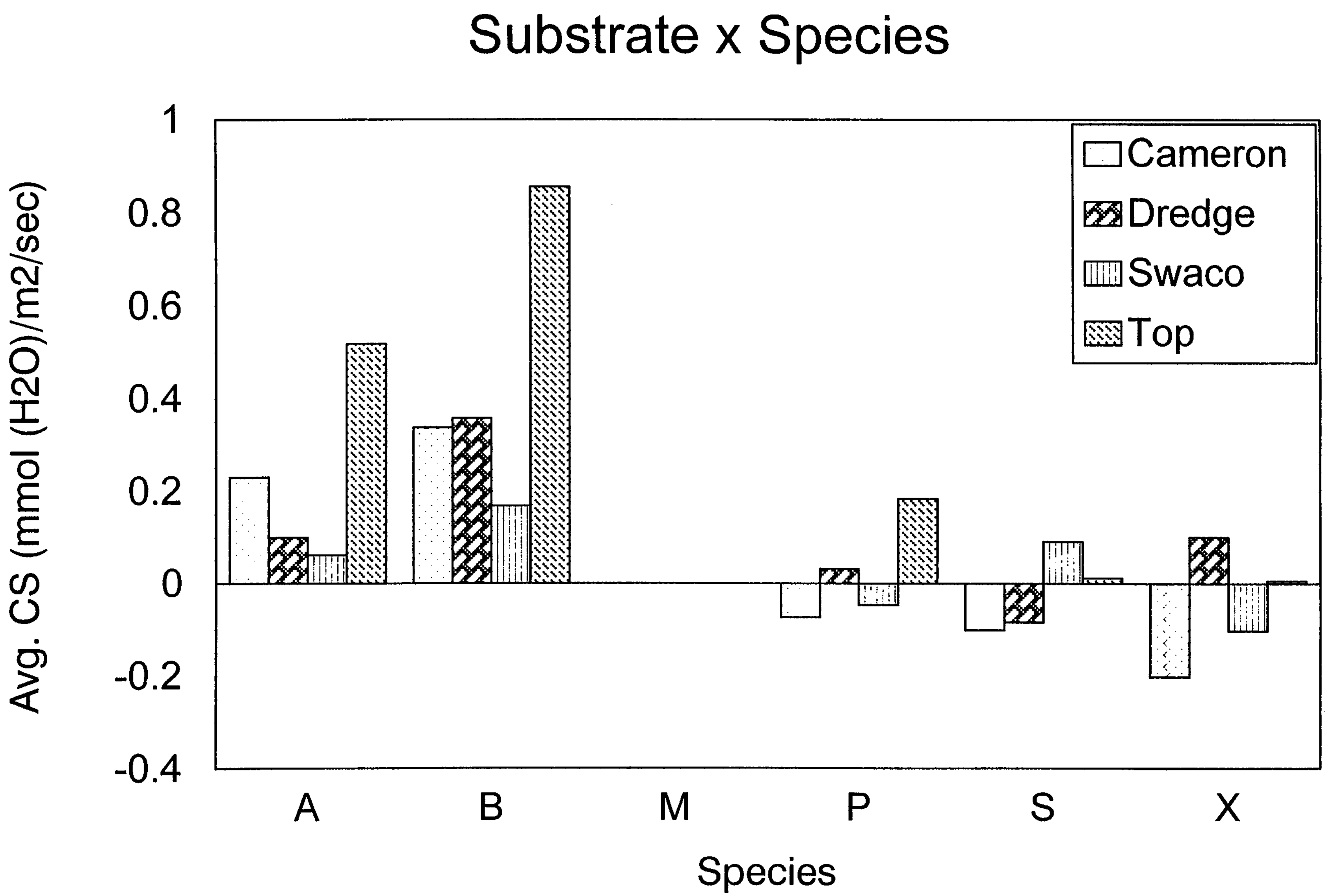


Figure 5. Interaction of plant species (A = elephantsear, B = bulltongue, M = mudflat, P = maidencane, S = average of three genotypes of wiregrass, X = average of the other three genotypes of wiregrass) and substrate type on stomatal conductance.



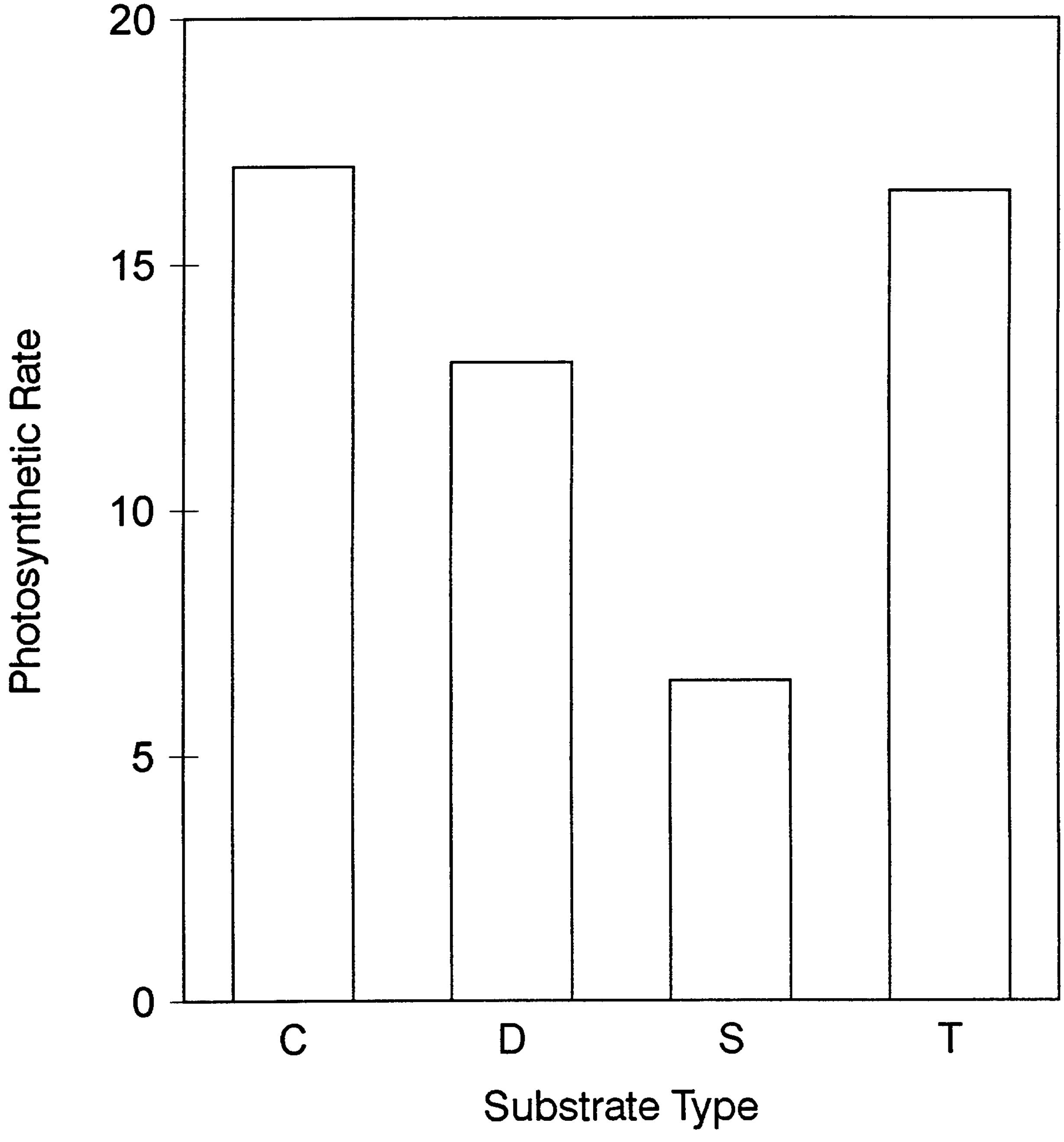


Figure 6. Overall photosynthetic response for the different substrate types (C = Cameron, D = Cameron capped with 20cm of dredge spoil, S = Swaco, T = topsoil).

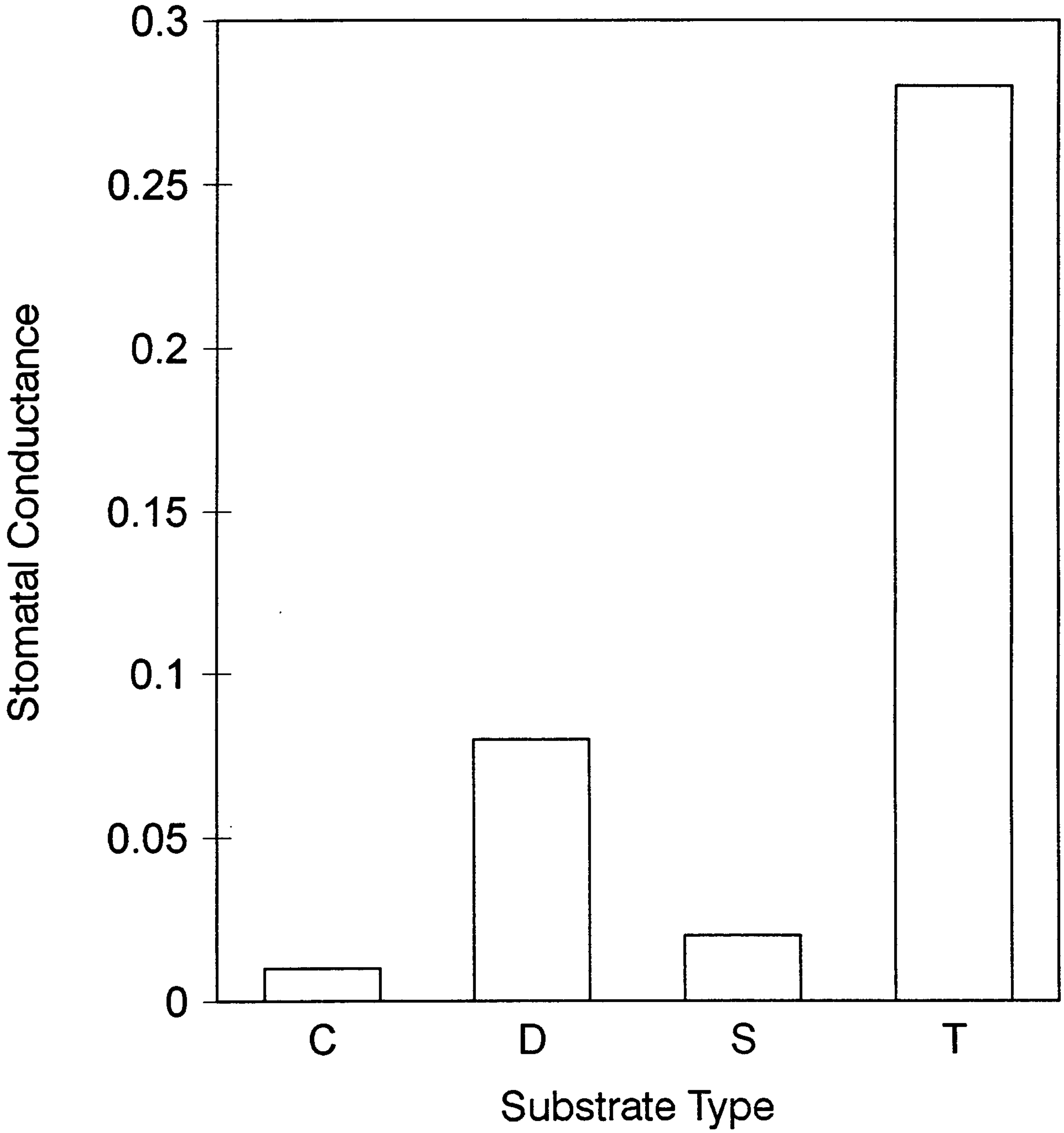


Figure 7. Overall stomatal conductance response for the different substrate types (C = Cameron, D = Cameron capped with 20cm of dredge spoil, S = Swaco, T = topsoil).

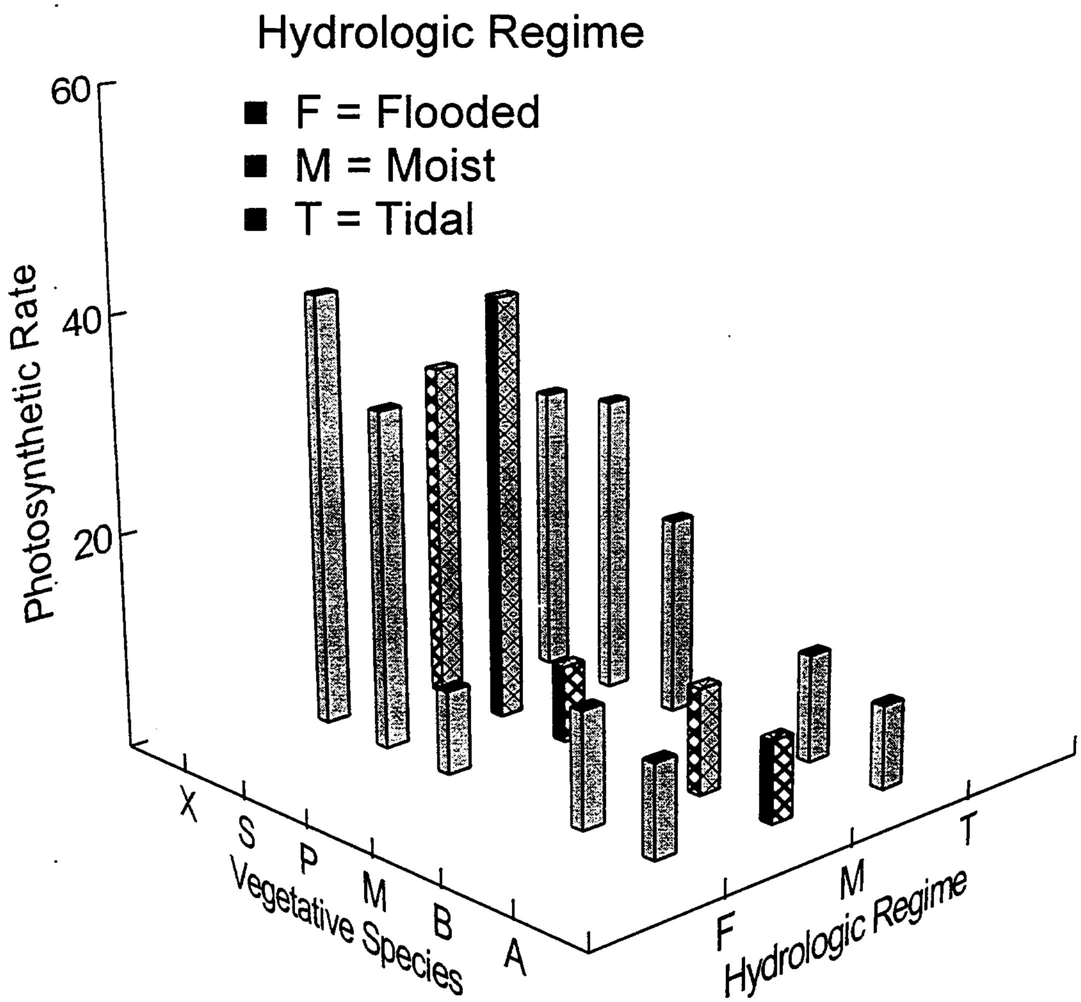


Figure 8. End of the season photosynthetic rate for the interaction between plant species (A = elephantsear, B = bulltongue, M = mudflat, P = maidencane, S = average of three genotypes of wiregrass, X = average of the other three genotypes of wiregrass) and hydrologic regime (F = flooded permanently, M = moist but not flooded, T = tides daily).

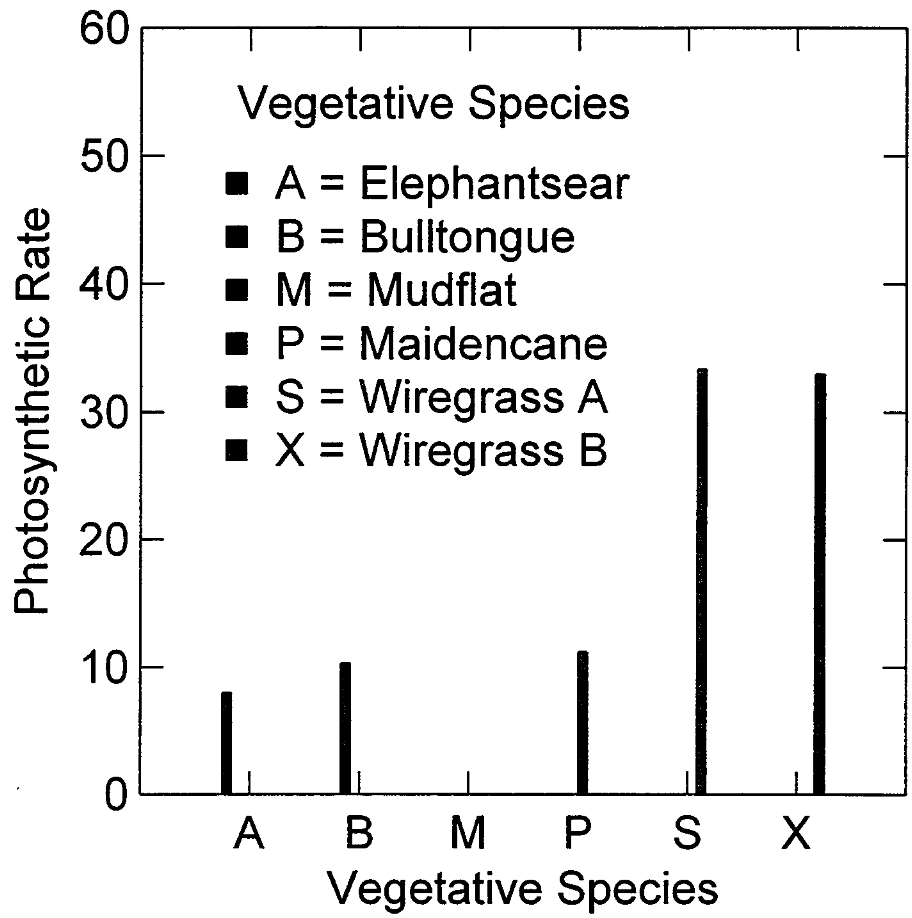


Figure 9. End of the season photosynthetic rate for the main effect of vegetative species.

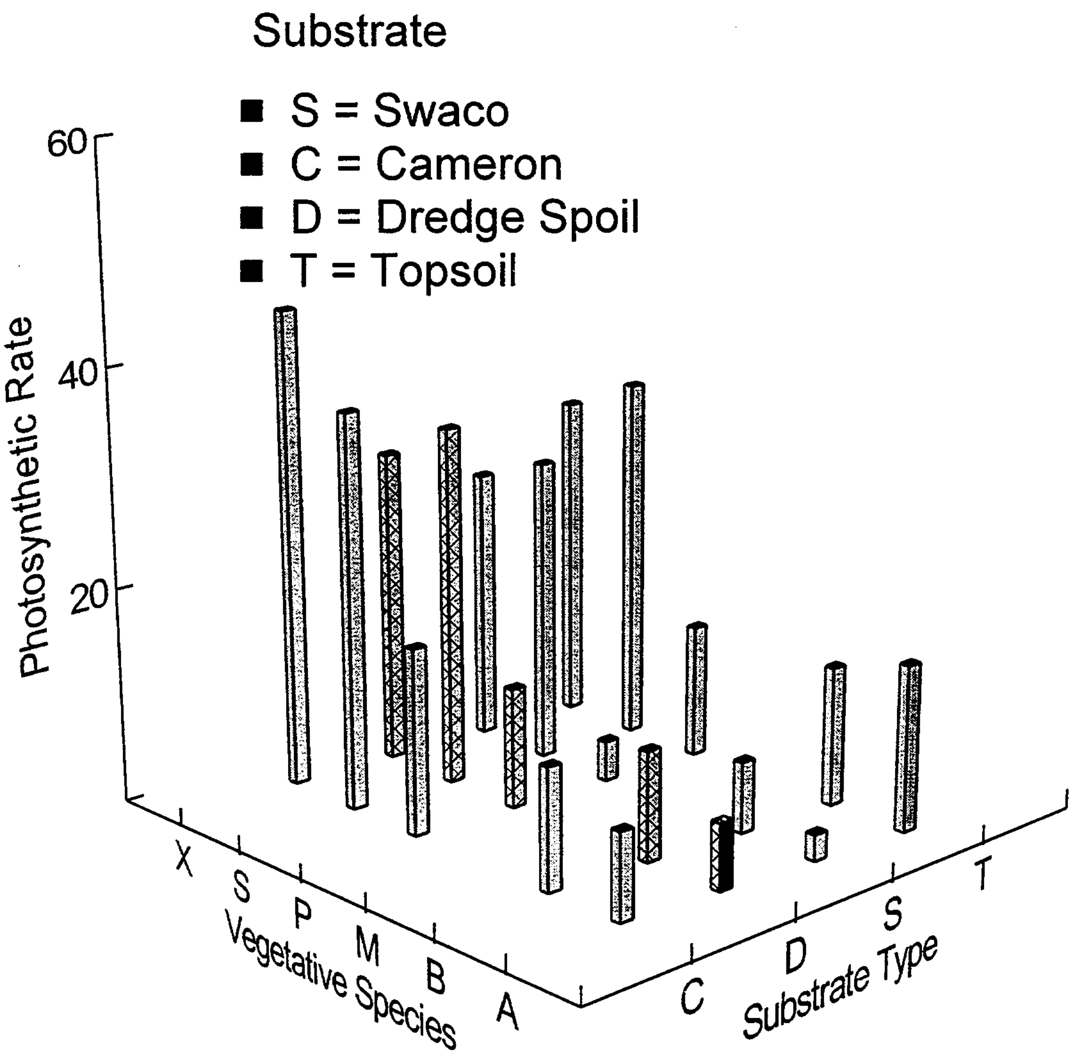


Figure 10. End of the season photosynthetic rate for the interaction between plant species (A = elephantsear, B = bulltongue, M = mudflat, P = maidencane, S = average of three genotypes of wiregrass, X = average of the other three genotypes of wiregrass) and substrate type.

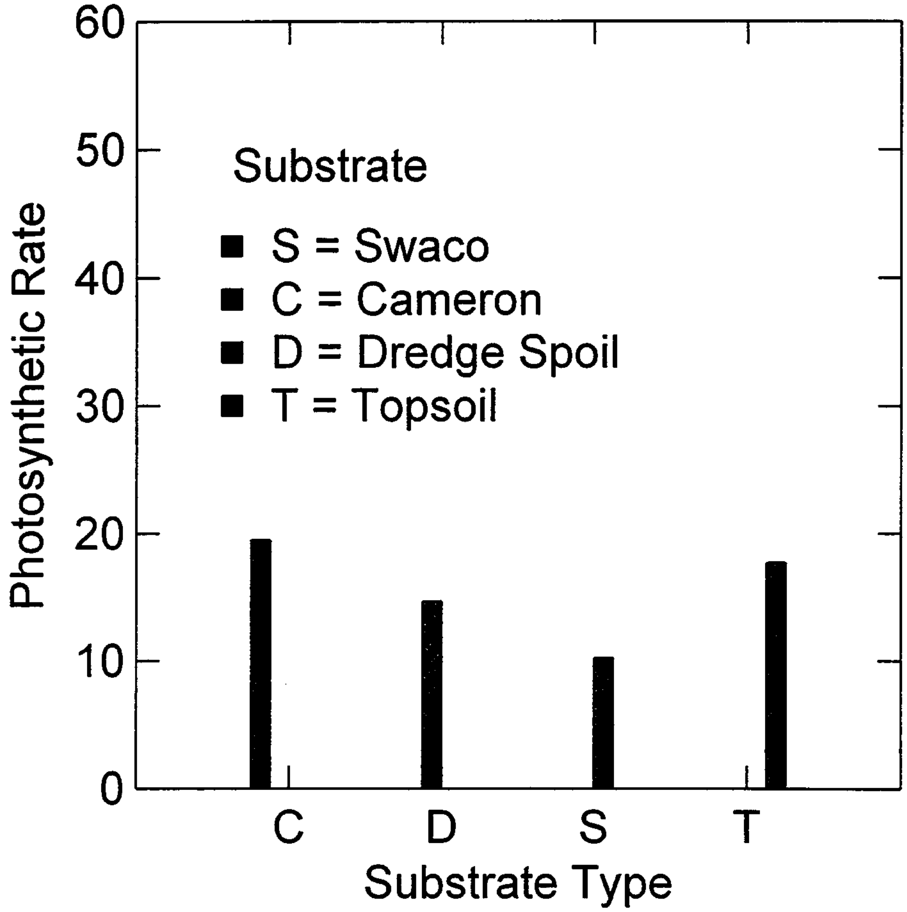


Figure 11. End of the season photosynthetic rate for the main effect of substrate type.

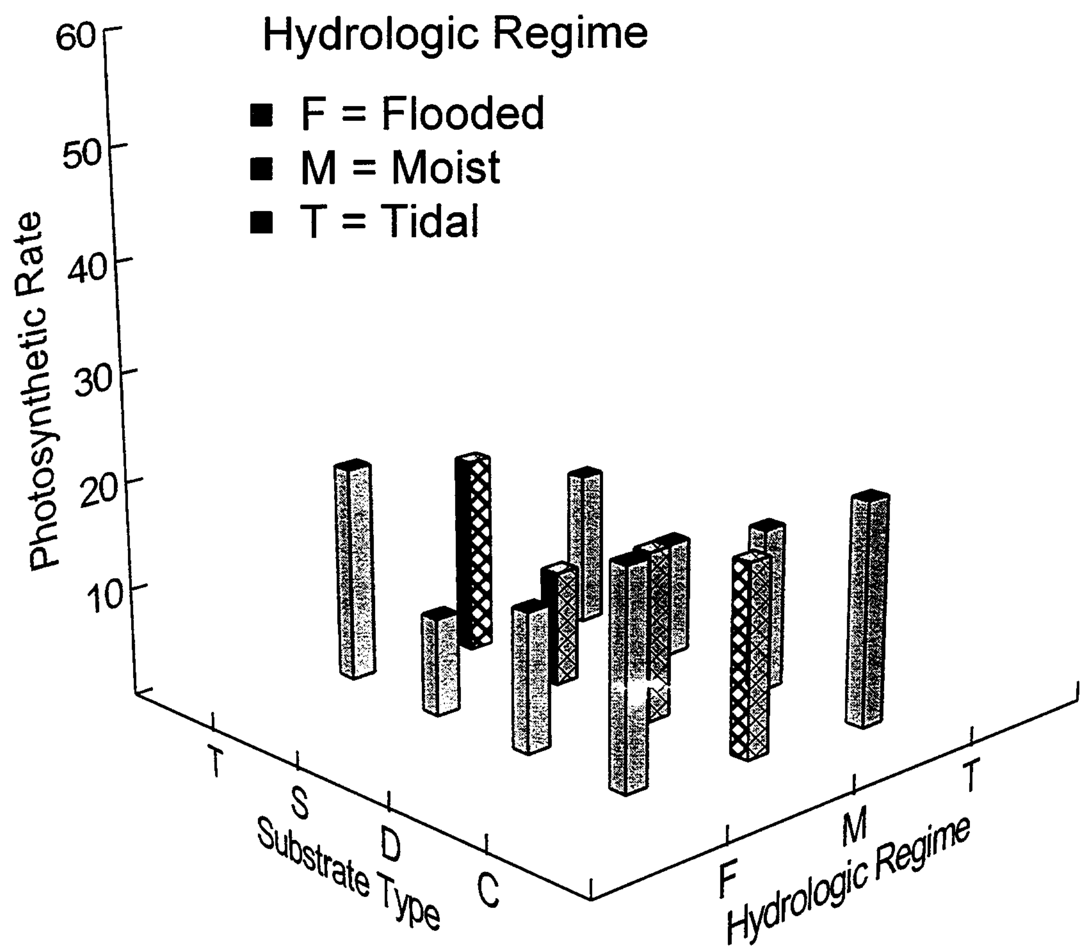


Figure 12. End of the season photosynthetic rate for the interaction between substrate type and hydrologic regime (F = flooded permanently, M = moist but not flooded, T = tides daily).

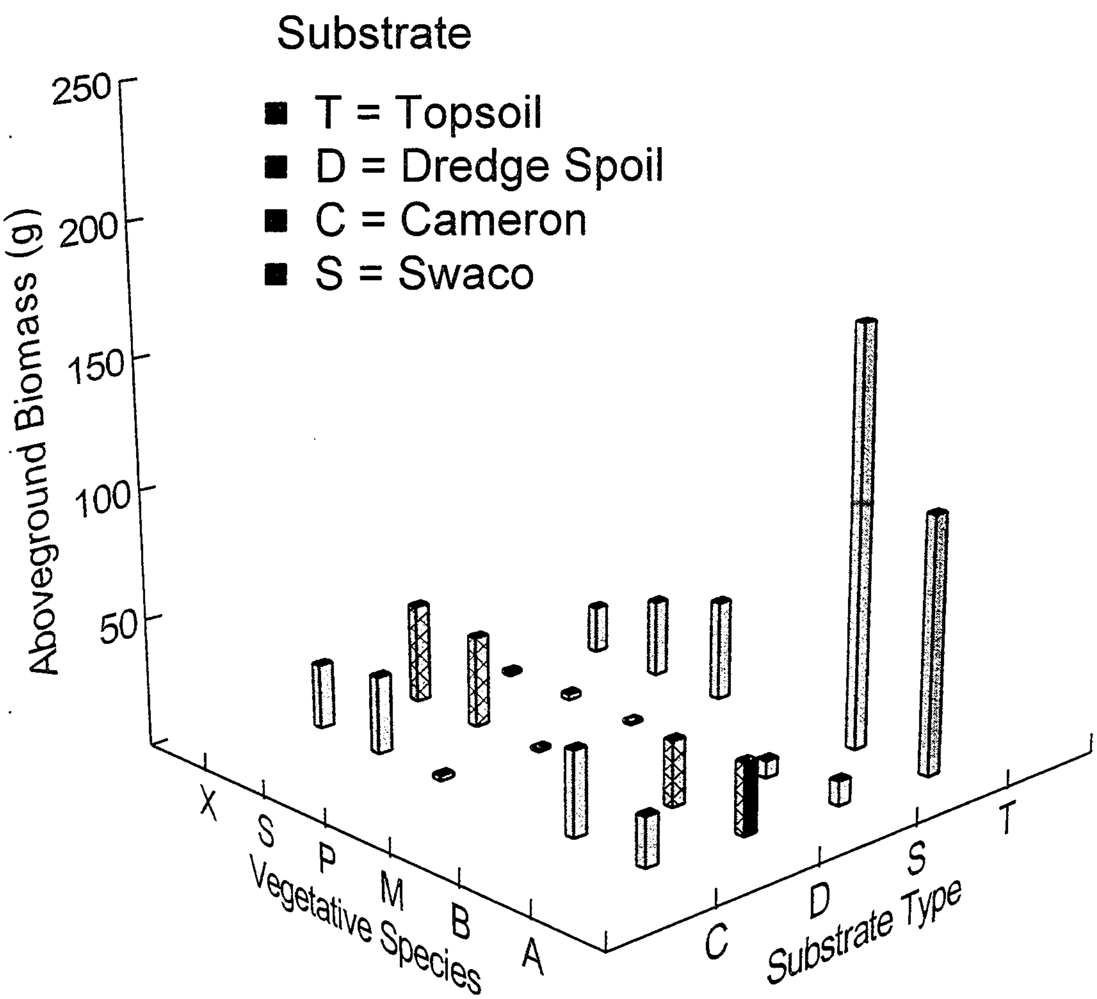


Figure 13. End of the season aboveground biomass production for the interaction between vegetative species (A = elephantsear, B = bulltongue, M = mudflat, P = maidencane, S = average of three genotypes of wiregrass, X = average of the other three genotypes of wiregrass) and substrate type.

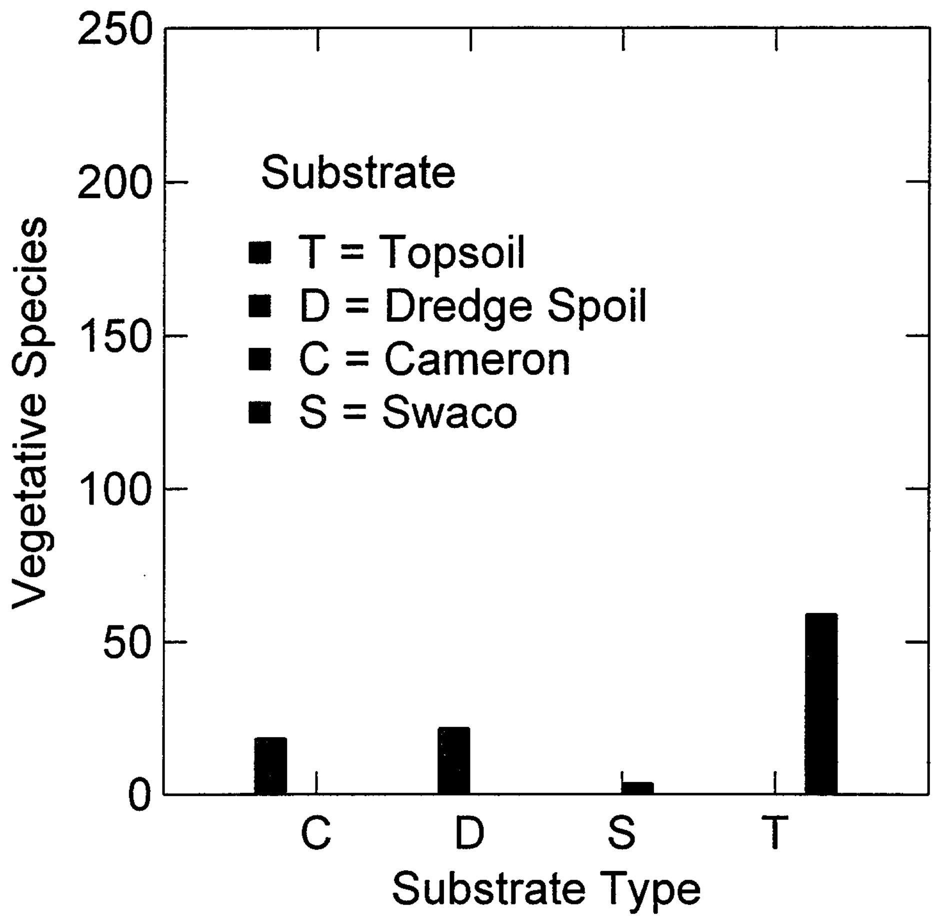


Figure 14. End of the season aboveground biomass production for the main effect of substrate type.

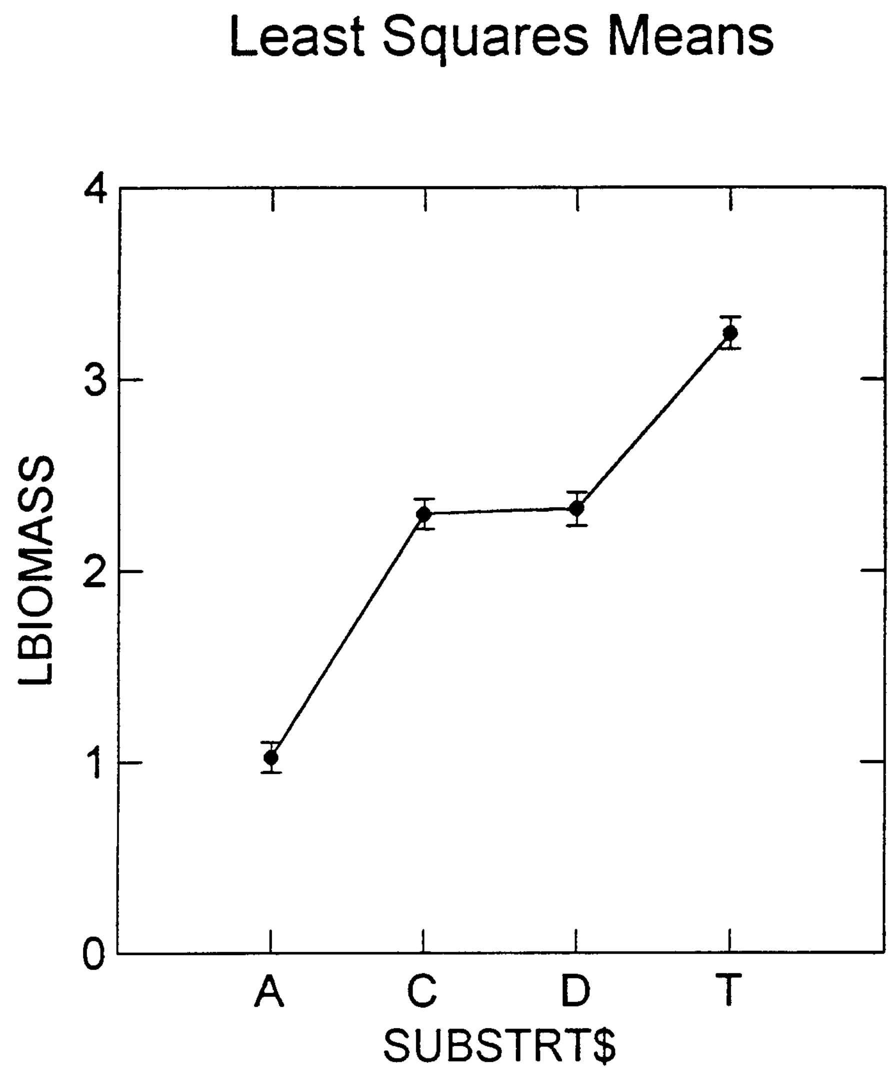


Figure 15. End of the season aboveground biomass production ($\log_e$) least squares means and least squares standard errors for the main effect of substrate type.

Appendix A

Background:

A study at the Wetland Biogeochemistry Institute of Louisiana State University (Kelly and Mendelssohn, 1994) demonstrated that restored sediments can support emergent wetland vegetation, albeit at lower rates of productivity than organic or natural wetland soils. This pilot study was quite informative, but limited in scope and, as such, several government agencies (US fish and Wildlife Service, National Marine Fisheries Service, Louisiana Department of Wildlife and Fisheries, US Army Corps) indicated that any extrapolation from this pilot study to a field study would be a serious error. The results from our intensive mesocosm project will provide a more accurate representation of actual field conditions.

Project Description:

Southeastern Louisiana University is unique in the United States in that it houses two mesocosm facilities designed for projects involving wetlands. Each of these mesocosm facilities contain one hundred forty-four 200-liter vessels, fully networked to four 3000-liter fiberglass supply reservoirs which enable homogeneous application of a particular treatment (e.g., salinity, nutrient level) for up to 36 of the mesocosms. The facilities are capable of simulating hydrologic conditions ranging from stagnant, to continuous circulation (riverine), and from average to episodic tidal events. The individual mesocosms are large enough to house a variety of wetland plants, and deep enough for natural root and shoot development of herbaceous vegetation. Because of their size, they provide results that should extrapolate to field conditions with far greater accuracy than traditional small-pot greenhouse experiments. Furthermore, the insulated mesocosms provide natural soil-temperature profiles enabling soil metabolism to also emulate field conditions.

The purpose of this study is to demonstrate that restored drill cuttings, a byproduct of the petroleum industry, can be safely used in coastal as well as inland wetland restoration projects. Prior to conducting laboratory experiments, composite soil samples of the recycled sediments were analyzed for pH and heavy metal concentrations.

Goals of this project:

The first goal was to determine if a fully functional wetlands mesocosm facility (i.e., a controlled system scaled between laboratory and field) would yield more natural information than that of traditional small-pot greenhouse experiments.

The second goal was to determine if wetland vegetation could be established on the restored cuttings; five species (including six different genotypes of wiregrass (*Spartina patens*) have been established.

The third goal involved the elemental analysis of (a) the cuttings material, (b) interstitial water, (c) aboveground water, and (d) plant tissues grown in the mesocosms.

The fourth goal involved toxicity testing using the mysid shrimp toxicity test (US EPA, 1993).

The fifth goal involved comparison of instantaneous rates of photosynthesis and conductivity of vegetation growing in cuttings with control vegetation, under three hydrologic regimes.

Products developed. We isolated the conditions necessary to create wetlands using restored drill cuttings. Hopefully, if year two of this project confirms the results of year one, and a field demonstration project yields similar results, rather than adding the innocuous material to hazardous waste sites, it will be used to enhance establishment of wetland vegetation in otherwise open-water systems.

The Mesocosm Facility:

The mesocosm facility itself consists of 144 mesocosm units which are 200-liter industrial strength polypropylene barrels networked by PVC pipe to 3000 liter fiberglass supply reservoirs (Figure 1). The ground water level within each mesocosm is externally controlled and different substrates are included as treatments. Each mesocosm contains approximately 150 liters of soil. There are four soil treatments in this experiment: Swaco-processed drill cuttings material, Cameron-processed drill cuttings material, Cameron substrate capped with 40 cm of dredge spoil, and topsoil. Each one of the four treatments is replicated in 36 mesocosm vessels. Each mesocosm has potential access to each supply tank. Acclimation of substrate within the mesocosm system occurred for two months prior to planting.

The Mesocosms:

Each of the 144 mesocosms are individually wrapped with one-half inch industrial grade black neoprene for insulation, then covered with heavy-duty cellophane to attach and seal the neoprene to that vessel. Each mesocosm is plumbed with two bulkhead fittings centered approximately 12.5 cm from the bottom of the mesocosm barrel and oriented approximately 45 degrees around the barrel from one another (Figure 2). One bulkhead fitting is attached to an adjustable, internal high tide plumbing arrangement and the other bulkhead fitting to an adjustable, internal low tide plumbing arrangement. The low tide plumbing arrangement consists of a 3/4 inch PVC elbow fitted to the inside surface of the bulkhead fitting, changing the orientation of the pipe from horizontal to vertical, the elbow is then fitted with a series of 3/4 inch PVC pipes which are jointed at distinct levels according to desired level for the low tide. The terminal piece of 3/4 inch PVC has approximately 100 1/8-inch holes drilled in it to allow water to enter the low tide drain but prevent the soil substrate from entering, since the low tide drains 10 cm below the soil substrate/air interface. The high tide bulkhead fitting is plumbed similarly, with the terminal piece of PVC pipe reaching approximately 20 cm higher than the sediment surface. The bulkhead fittings are attached externally to a series of pipes that will return the water to that vessel's final destination (i.e., one of the four reservoirs in the mesocosm facility)(Figure 3). Once each mesocosm is plumbed internally and its specific soil type added, an interstitial water catchment pipe is placed into the center of the mesocosm. The interstitial water catchment pipe consists of a two-foot piece of 3/4 inch PVC pipe with a cap on both the lower and upper end. The lower end of the pipe received a 1 mm slit10 cm vertically, and was then capped. This allows subsurface water to drain into the catchment tube. The upper portion of the catchment tube is capped and contains a 1/16 inch hole to prevent a pressure gradient from forming. The interstitial water catchment pipe is then inserted to a depth of 15 centimeters below the soil surface in the center of the mesocosm.

The Circulatory System:

The circulatory system of the mesocosm facility consists of two subsystems, namely the water delivery and recovery subsystem and the air delivery subsystem.

The Water Delivery and Recovery Subsystem:

Four 3000-liter fiberglass reservoirs form the basis of the water system. These reservoirs allow the delivery of four independent, water-soluble treatments to as many as 36 individual mesocosms each. In this particular instance, they represent each of the four substrate treatments: Swaco, Cameron, dredge spoil, and topsoil. The system is gravity-fed from the supply tanks to stand-pipes located between each series of four mesocosms (Figure2 and Figure 4). From the standpipes, solutions are fed to each mesocosm via the air delivery system.

The Air Delivery Subsystem:

The air lift portion of the mesocosm facility circulatory system consists of three Sweetwater™ regenerative blowers connected to 2 inch PVC pipe spanning each row of mesocosm units. At each series of standpipes, airline tubing provides the conduit for air to lift fluid from the standpipe to each mesocosm (Figure 4). Fine adjustment of air via brass valves allows the precise control of the amount of fluid lifted from each standpipe. In this way adjustments were made for tidal conditions versus moist-but-unflooded (Figure 5).

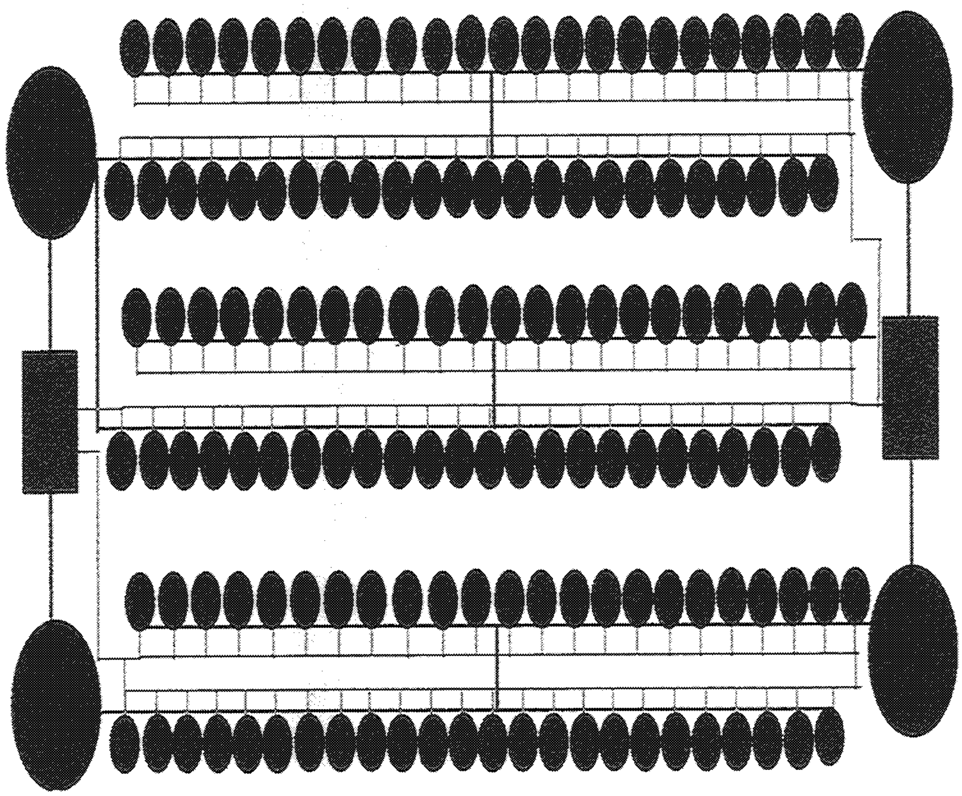


Figure 1. Diagram of mesocosm system containing 4 supply tanks, 144 two-hundred liter mesocosms, and 2 delivery stations with blowers, timers and switches. Mesososms are housed in a 90'x34' greenhouse, which are climate-controlled. NOTE: diagram does not represent actual experimental design or pipe layouts.

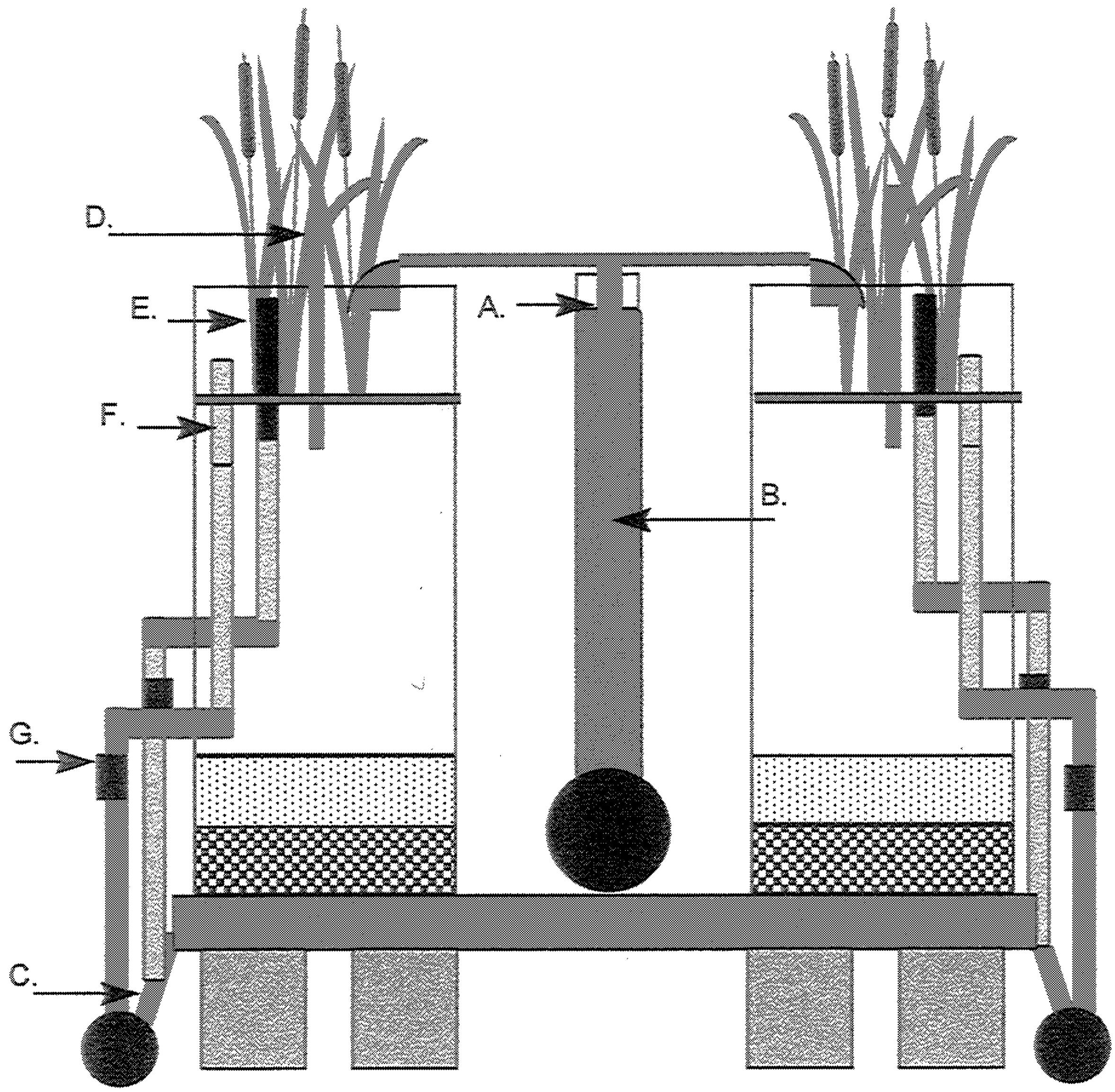


FIGURE 2. ONE PAIR OF MESOCOSMS WITH (A.) SUPPLY WATER STAND PIPE, (B.) WATER LIFTED BY VERTICAL AIR STREAM, AND (C.) GRAVITY RETURN TO SUPPLY RESERVOIRS. TIDE LEVELS ARE REGULATED BY EITHER (E.)HIGH TIDE OVERFLOW OR (F.)LOW TIDE OVERFLOW. SOIL WATER SAMPLING IS FACILITATED BY (D.) INTERSTITIAL WATER CATCHMENT PIPE. RATE OF TIDAL DRAINAGE IS CONTROLLED BY (G.) BALL VALVES

Figure 3

Mesocosm layout showing individual mesocosm units, ball valves for regulationg tidal drain rates, and pipes for drain water returning to supply reservoirs.

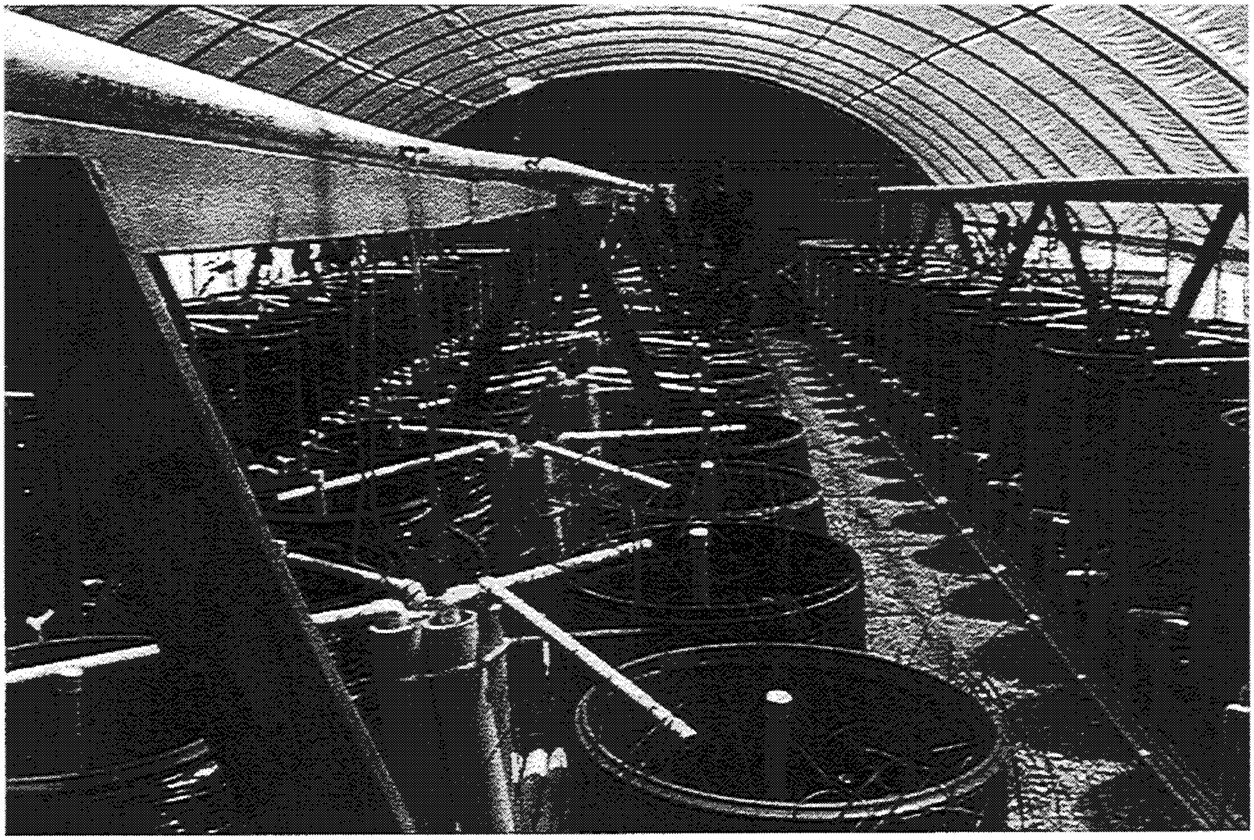


Figure 4

Top view of mesocosm facility showing supply standpipes (groups of four), overhead air supply lines, and airline tubing leading to water lift tubes. Capped vertical pipes in each mesocosm are catchment tubes for interstitial water samples.

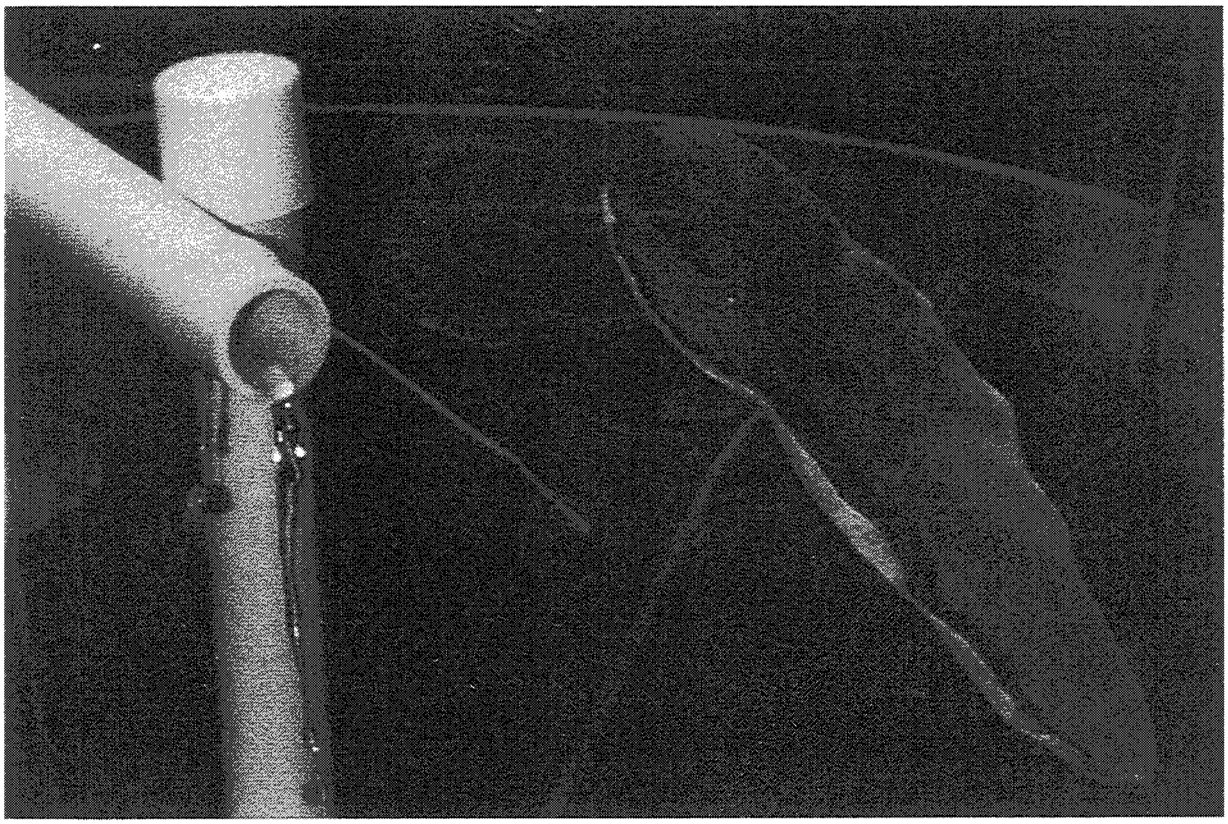


Figure 5

View of individual mesocosm unit showing tidal influx controlled by timers and regenerative blowers. Capped interstitial water catchment pipe is in background.